USNRDL-TR-67-4
20 December 1966

INCREASED RADIATION RESISTANCE OF MICE INJECTED WITH BEE VENOM ONE DAY PRIOR TO EXPOSURE

by
W.H.Shipman
L.J.Cole

U.S. NAVAL RADIOLOGICAL DEFENSE LABORATORY
SAN FRANCISCO · CALIFORNIA · 94135

EXPERIMENTAL PATHOLOGY BRANCH
L. J. Cole, Head

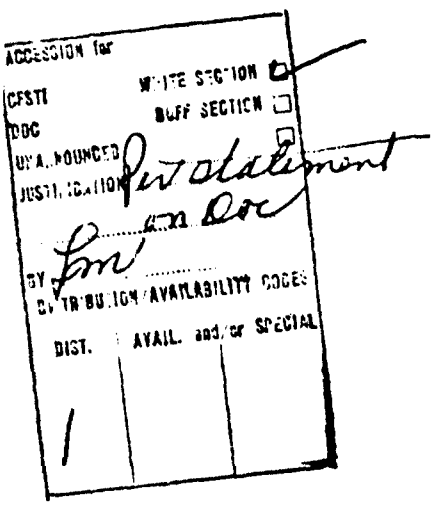
BIOLOGICAL AND MEDICAL SCIENCES DIVISION
E. L. Alpen, Head

ADMINISTRATIVE INFORMATION

This work was accomplished under the Bureau of Medicine and Surgery Work Unit MR005.08-0006, as described in the U.S. Naval Radiological Defense Laboratory Annual Report to the Bureau of Medicine and Surgery (DD Form 1498) of 31 December 1965. This study was supported through funds provided by the Bureau of Medicine and Surgery.

DDC AVAILABILITY NOTICE

Distribution of this document is unlimited.



Eugene P. Cooper
Eugene P. Cooper
Technical Director

D. C. Campbell, CAPT USN
Commanding Officer and Director

ABSTRACT

Mice were injected with bee venom dissolved in a 0.90% NaCl solution. This injection was given either intraperitoneally or subcutaneously 24 hours before the mice were irradiated with X rays. It was found that, after exposure to a lethal dose of radiation (800 - 850 R) the venom-injected mice had a consistently higher number of survivals than the controls, and that the subcutaneously-injected mice had a higher number of survivals than the intraperitoneally-injected mice. The question as to whether this radioprotective effect of bee venom is due to its general stress-like effect, or to the action of a specific chemical component has been discussed.

SUMMARY

The Problem:

In the search for new radioprotective agents, the question arose whether the known multiple pharmacologic and physiological effects of bee venom would afford protection against lethal X-ray exposures in mice. Since bee venom acts as a stressing agent, and since certain stressors had been shown in the literature to confer a degree of radioprotection, it was considered of interest to test the hypothesis that administered bee venom would be radioprotective.

The Findings:

Bee venom has a significant radioprotective effect in mice exposed to X-radiation in the lethal dose range. It was found that if mice were injected subcutaneously 24 hours before X irradiation (850 R) a larger percentage would survive by 30 days than if they were injected intraperitoneally, or if saline were injected. There are indications that the radioprotection may not necessarily be caused by the stressing action of the venom, but rather by one of its components.

INTRODUCTION

It is known that the response of animals to whole-body X irradiation in the lethal range can be modified by certain changes in their physiological state induced prior to exposure. For example, the administration of estrogens (1), bacterial endotoxin (2), colchicine (3), urethan (4) to mice 1 day or more before lethal irradiation, results in a modest but definite increase in 30-day survival. The mechanism(s) by which these diverse agents enhance the radiation resistance of rodents is not clear; however, it can be stated that their protective effect must be mediated via modes of action different from those of the 'classical' chemical radioprotectors such as cysteine, cysteamine or AET, which are only effective when administered immediately prior (of the order of 1/2 hour) to irradiation.

Considerations of the chemical composition and useful pharmacological effects of the known components of bee venom suggested to us the possibility that it might be of value as a radioprotective agent. It was thought that the venom might produce a degree of physiological 'stress' in animals, and thereby elicit a neuroendocrine response (pituitary-adrenal stimulation)-the so-called adaptation syndrome (5,6), which would increase radiation resistance. There are, in fact, some suggestions in the literature (cf. 7) that stimulation of adrenal cortical activity by injections of salicylate, bacterial toxins, ACTH, or by exposure to cold will increase slightly the resistance of animals to whole-body irradiation.

The physiopathologic action of bee venom is known to exhibit three general manifestations: neurotoxic, hemorrhagic and hemolytic. Bee venom is a complex mixture of enzymes, toxins, and several unidentified substances. The active constituents in bee venom include the enzymes phospholipases A and B (8), hyaluronidase (9), a powerful surfactant (10), a lactic acid dehydrogenase inhibitor (11), and the hemolytic and neurotoxic polypeptides melittin (12) and apamine (13). The two latter substances constitute the largest fraction by weight of bee venom. Histamine is present at approximately 1% concentration, and in addition it is released by the tissue during bee venom poisoning (14). The injection of bee venom into animals has also been shown to result in a profound hypothermia (15).

For a preliminary test of this hypothesis, groups of mice in this study received a single injection of bee venom prior to being exposed to X radiation in the lethal range, and the rate of 30-day mortality was noted. A significant increase in radiation resistance was found.

MATERIALS AND METHODS

The experimental animals used were genetically homogeneous LAF_1 hybrid male mice, 12 to 16 weeks of age, from the NRDL colony. The estimated X-ray LD_{50} for these mice is 740 R. The X-radiation source was a Westinghouse Therapy Unit. The radiation factors were: 250 kvp; 15 ma; 0.5 mm Cu plus 1 mm Al filters; skin-to-target distance 100 cm; exposure rate 29 R/min as measured in air with a Victoreen R-meter. The irradiation was given as a single exposure. The mice were placed in perforated 50 ml Lusteroid centrifuge tubes, radially positioned around a circular wooden turntable and rotated at 3.5 rpm directly under the X-ray tube. Following radiation exposure, the mice were housed in cages, 10 per cage, and were observed daily for mortality over a period of one month. Purina Chow pellet feed was available ad libitum.

Three bee venoms were used in this study: A Summer, a Winter, and a Spring venom. The venom used in the first experiment (Figure 1) was collected August 29, 1965 in Ithaca, New York, by Dr. Roger A. Morse, Associate Professor of Apiculture at Cornell University. The second venom used (Figure 2) was collected April 2, 1966 by the senior author in Santa Cruz, California; the third preparation used (Figure 3 and dimethyl sulfoxide experiments) was collected April 1966 by Mr. Charles Mraz of Middlebury, Vermont. No differences in toxicity for mice among the three venoms could be detected.

RESULTS

In the first experiment one group of 11 male mice (4 months old) received 1.24 µg of bee venom (dissolved in isotonic saline) per gram of body weight. A control group of 9 mice received the equivalent volume of saline only. The injections were delivered intraperitoneally 24 hours before exposure to 800 R of X radiation. At the end of the 30-day observation period (see Figure 1) 64% of the mice that received the bee venom were alive, while only 22% of the control group had survived ($P = 0.005$).

The next experiment was designed to estimate the relative degree of protection afforded by two different routes of injection of the bee venom. Each of a group of 10 mice was injected subcutaneously with 5.6 µg of bee venom per gram and a second group received 1.1 µg per gram intraperitoneally. A third group of 14 mice was injected intraperitoneally with the same volume (0.2 ml) of saline only. (A preliminary toxicity trial had shown that these doses of venom were at the threshold of the toxic, i.e., lethal, dose in mice, administered

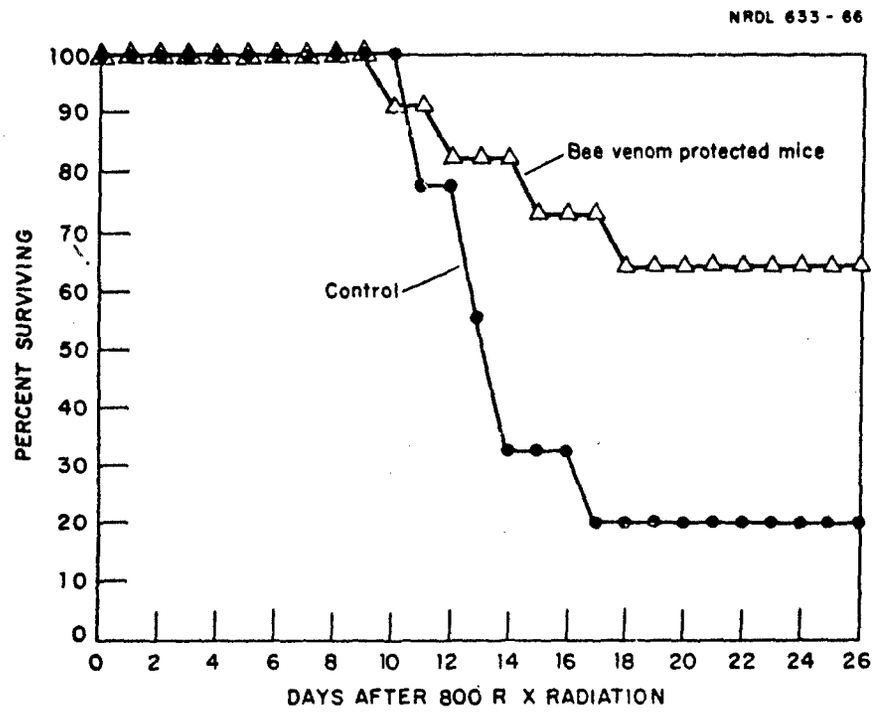


Figure 1. Mortality response of X-irradiated mice (800 R) each receiving 1.24 μg bee venom per gram body weight (ip) 24 hours before exposure, as compared with that of saline controls

by each of these injection routes.) Twenty-four hours later, all three groups were exposed together to 825 R of X radiation. As can be seen in Figure 2, all of the control group died, while about 20% of the intraperitoneally injected group and 80% of the subcutaneously injected groups were alive at 30 days. ($P < 0.001$ for subcutaneously injected mice versus saline controls.)

In the next experiment, the effect of higher X-ray doses was evaluated. One group of mice received 1.19 μg of bee venom per gram given intraperitoneally 24 hours before exposure to 850 R, while the controls received a similar volume of saline. The interpretation of the data is made difficult by the unexplained early deaths of the venom-treated mice (see Figure 3). However, it is believed that these early deaths resulted from bacterial contamination of the venom. The validity of this belief was examined by filtering a sample of the venom solution through a 0.45 μ Millipore filter to remove any bacteria and repeating the experiment described in Figure 3 with the filtered venom. No early deaths were observed with the filtered venom and the mortality statistics remained the same.

Effect of Dimethylsulfoxide as a Solvent for Bee Venom. In an attempt to study the effect of increasing the rate of absorption of bee venom, venom solutions were prepared in dimethyl sulfoxide (cf. 16, 17) and injected into the mice. Two solutions were prepared each containing 1.07 mg of bee venom in a total volume of 25 ml. One solution was 20% v/v dimethyl sulfoxide: 0.90% sodium chloride, the other 60% v/v of the same mixture. Five populations of LAF_1 mice were selected: Group I (10 mice) received 0.86 μg of bee venom (no dimethyl sulfoxide) per gram intraperitoneally. Group II (10 mice) was injected subcutaneously with 4.3 μg of bee venom (dimethyl sulfoxide-free) per gram. The third Group received subcutaneously 4.2 μg of bee venom (dissolved in 20% dimethyl sulfoxide) per gram. Group IV were injected subcutaneously with 4.1 μg of bee venom (dissolved in 60% dimethyl sulfoxide) per gram. Group V received saline only. All five groups received a dose of 850 R X radiation 24 hours after the bee venom injection. For these experiments, the mice were housed in small hanging cages, 2 per cage, and their drinking water contained Polymyxin B (840 units/ml) and Neomycin (100 mg %).

The results of the dimethyl sulfoxide-bee venom injections were as follows: After the 16th day postirradiation all controls and dimethylsulfoxide-treated mice were dead. The 60% dimethyl-sulfoxide-treated mice (Group IV) died earlier than the saline control group (V), while the 20% dimethyl sulfoxide-treated mice (Group III) died at a slower rate than the saline control group. The mice which had received bee venom dissolved in saline showed a 30-day survival of

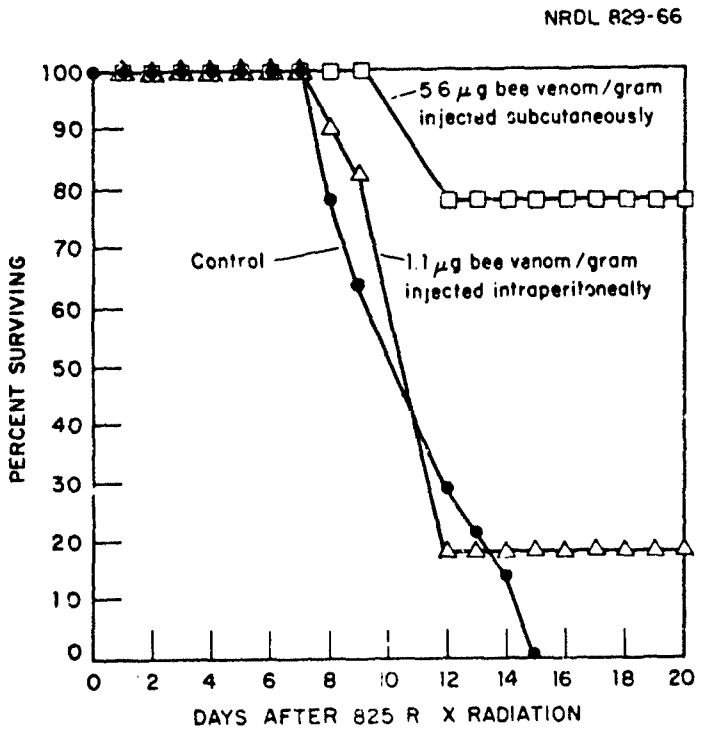


Figure 2. Comparison of effectiveness of bee venom injected intraperitoneally or subcutaneously in modifying the response of mice to a lethal dose of X radiation (825 R). Venom administered 24 hours prior to exposure.

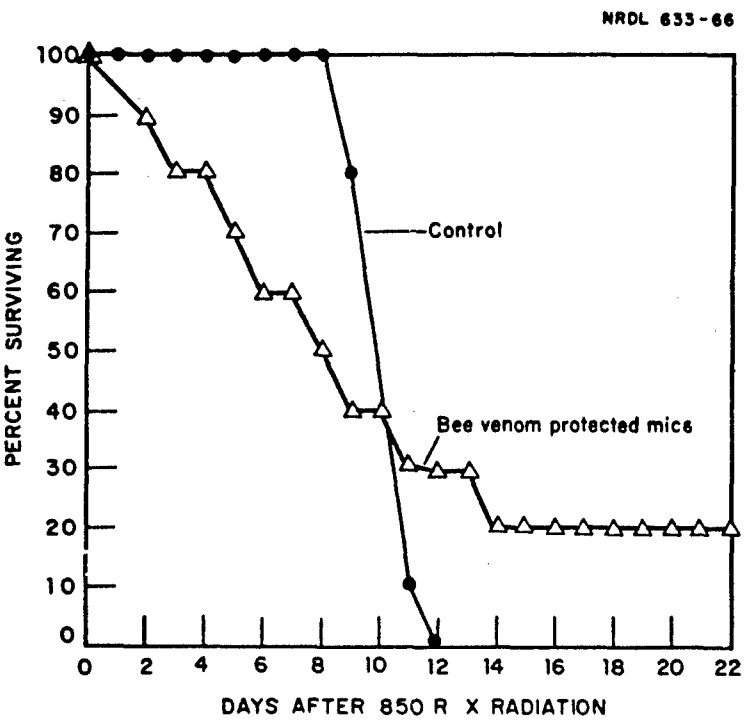


Figure 3. Effect of intraperitoneal injection of bee venom (1.19 μg per gram) 24 hours prior to X irradiation (850 R) of mice. The early deaths in this experiment was found to be due to bacterial contamination of the venom preparation.

70% for those receiving the venom subcutaneously (Group II), and 20% for the group in which the venom was injected intraperitoneally (Group I). These results confirm the earlier observations that the venom injected subcutaneously affords greater protection than that injected intraperitoneally.

DISCUSSION

These experiments with bee venom administered to mice 1 day prior to X irradiation, clearly show that a significant increase in radiation resistance was afforded, in terms of 30-day survival. The results are perhaps more significant when it is realized that no attempt was made to optimize the parameters of the experiments.

At this time, sufficient information is not available upon which to base a definitive mechanism for the radioprotective effect of bee venom. The fact that protection obtains when venom is administered 24 hours before radiation exposure places it in a different category from cysteine, AET and related compounds, which must be administered just prior (ca. 30 minutes) to irradiation in order to be effective.

The most reasonable assumption at present is that bee venom exerts a stressor-like action, in view of the fact that it elicits hypothermia (15), and produces an inflammatory response and fever. It is to be noted that Bacq, in his recent monograph on chemical protection against radiation (18) dismisses the lowered body temperature as a factor of importance in chemical radiation protection, and states that "the stimulation of the adrenal cortex has no significance for radioprotection..." However, the constellation of nonspecific neuro-endocrine reactions to a stress which Selye has termed the adaptation syndrome, is very complex, and is not easily amenable to quantitative measurement; it is likely that the 'degree of stress', and its timing relative to radiation exposure, are critical determinants as to whether the elicited response is radioprotective, or indeed, deleterious. If the venom does act as a stressing agent it compares favorably with other known stressors. Thus, when X rays were used as a stressing agent (6), 36% of the treated mice survived at a dose of 700 R (an LD_{99}+) delivered under optimized conditions. When cold was used as the stress (1 hour in the refrigerator every day for 7 to 30 days), 15% of the mice survived 700 R X rays (an LD_{99} + under these conditions). When a chemical stressor such as sodium salicylate (0.4-0.6 mg per gram) was administered daily for 12 days, 25% of the mice survived an otherwise lethal dose of X rays (6). As can be seen from Figures 1 - 3, the protection afforded by a single administration of bee venom 24 hours before irradiation affords much greater protection than did the stressing agents reported in the literature.

The observation that greater radioprotection was obtained the mice when the venom was injected subcutaneously than when injected intraperitoneally suggests the following question: Is the larger amount of venom responsible for the difference in the degree of radioprotection or is the difference the result of the rate of absorption? Since the amount received intraperitoneally cannot be increased without toxic killing of the mice, it was felt that the use of dimethyl sulfoxide to increase the rate of absorption of venom injected subcutaneously might answer this question. Since there was no visible or suspected physicochemical reaction between the venom and dimethyl sulfoxide when mixed, the data suggest that the presumed increase in absorption rate is the explanation of the higher mortality rate.

It would be of value to fractionate bee venom into its several components and evaluate each for its radioprotective effect. If the protection is found to be due to a nontoxic fraction of the bee venom, the stress theory of action would have to be eliminated or modified. On the other hand, the finding of one or two protective fractions which are also potent stressors would be of great interest. Such studies may lead to a better understanding of the physiological mechanism(s) by which bee venom affords protection against radiation lethality in mice, and to a further clarification of the role of stress and the adaptation syndrome.

REFERENCES

1. Straube, R. L., Patt, H. M., and Swift, M. N. Amer. J. Physiol. 155: 471, 1948.

2. Smith, W. W., Alderman, I. M., and Gillespie, R. F. Amer. J. Physiol. 191: 124, 1957.

3. Smith, W. W. Science 127: 340, 1958.

4. Cole, L. J. and Gospe, S. R. Radiation Res. 15: 684, 1961.

5. Selye, H. The Physiology and Pathology and Exposure to Stress. (Acta Inc., Montreal 1950.) Supplements 1951 and 1952.

6. Bacq, Z. M. and Alexander, P. Fundamentals of Radiobiology, 2nd ed., (1961, Pergamon Press, New York).

7. Betz, E. H. Contribution a l'etude du Syndrome Endocrinien Provoque par l'irradiation Totale de l'organisme, (Masson, Paris, 1956).

8. Doery, Hazel M. and Pearson, Joan E. Biochem. J. 92: (3) 599, 1964.

9. Baker, S. H., et al. Nature 199: 693, 1963.

10. Haydak, M. H. Bee Venom. (The State of Iowa Report of the State Apiarist for the year ending 31 Dec 1951, pp 105-126).

11. Fleckenstein, A. Arch. Exp. Path. Pharmak. 213: 265, 1951.

12. Kreil, G. Monatsh. Chem. 96:(6) 2061, 1965.

13. Habermann, E., Reiz, K. G. Biochem. Z. 341:(5) 451, 1965.

14. Rothschild, A. M. Brit. J. Pharmacol. 25:(1) 59, 1965.

15. Benton, A. W., Heckman, R. A., Morse, R. A., J. Appl. Physiol. 21:(4) 1228, 1966.

16. Horita, A. Life Sciences 3: 1389, 1964.

17. Kligman, A-M. J. Amer. Med. Assoc. 193: 796, 1965.

18. Bacq, Z. M., Chemical Protection Against Ionizing Radiation, (Charles C. Thomas, Springfield, Ill., 1965).

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
U. S. Naval Radiological Defense Laboratory
San Francisco, California 94135

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP

3. REPORT TITLE

INCREASED RADIATION RESISTANCE OF MICE INJECTED WITH BEE VENOM ONE DAY PRIOR TO EXPOSURE

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5. AUTHOR(S) *(First name, middle initial, last name)*
Shipman, William H.
Cole, Leonard J.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
2 March 1967	19	18

8a. CONTRACT OR GRANT NO.

b. PROJECT NO.

c. BuMed Work Unit MR005.08-0006

d.

9a. ORIGINATOR'S REPORT NUMBER(S)
USNRDL-TR-67-4

9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*

10. DISTRIBUTION STATEMENT

Distribution of this document is unlimited.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
Bureau of Medicine and Surgery
Washington, D.C. 20390

13. ABSTRACT

Mice were injected with bee venom dissolved in a 0.90% NaCl solution. This injection was given either intraperitoneally or subcutaneously 24 hours before the mice were irradiated with X rays. It was found that, after exposure to a lethal dose of radiation (800 - 850 R) the venom-injected mice had a consistently higher number of survivals than the controls, and that the subcutaneously-injected mice had a higher number of survivals than the intraperitoneally-injected mice. The question as to whether this radioprotective effect of bee venom is due to its general stress-like effect, or to the action of a specific chemical component has been discussed.

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Bee venom X radiation protection Mice						